Determining the magnitude of early steps of fatty acid oxidation in glioma using ^{18}F-FPIA PET/MRI

Short title: Measuring fatty acid oxidation in gliomas using ^{18}F-FPIA PET/MRI

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Sponsor: Imperial College London is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance Manager at:

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Investigator Protocol Agreement Page

- I confirm agreement to conduct the study in compliance with the protocol.
- I acknowledge that I am responsible for overall study conduct.
- I agree to personally conduct or supervise the described clinical study in accordance with Good Clinical Practice requirements.
- I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations.

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1. Synopsis

Name of Sponsor: Imperial College London
Name of biomarker: ^{18}F-fluoropivalate positron emission tomography magnetic resonance imaging (^{18}F-FPIA PET/MRI)
Title of Study: Determining the magnitude of early steps of fatty acid oxidation in glioma using ^{18}F-FPIA PET/MRI
Study Centres: • Imanova Ltd, Hammersmith Hospital Campus, UK • Imperial College London • Imperial College NHS Trust, London
Phase of Development: Explorative biomarker use/ Phase 2
Primary Objective: • Determine the magnitude of the early steps of fatty acid oxidation in glioma using ^{18}F-FPIA PET/MRI. **Secondary objective:** • Compare magnitude of tracer-derived biochemical uptake measure with tumour grade. **Tertiary objective:** • Compare magnitude of tracer-derived biochemical uptake measure with ex vivo gene expression specifically for metabolism. • Compare magnitude of tracer-derived biochemical uptake measure with ex vivo tissue metabolite analysis using Mass Spectrometry (MS). • Compare the magnitude of tracer-derived biochemical uptake measure with ex vivo protein and phosphoprotein analysis from the major cell proliferation and survival pathways including phospho ERK 1/2 and pSer641. • Comparison of flux constants derived from mathematical modelling of ^{18}F-FPIA PET/MRI data with tumour grade. • Comparison of tracer-derived biochemical uptake measure with conventional MRI variables including perfusion imaging and contrast enhancement.
Study Design: Primary Endpoint: • Quantitative measurement of ^{18}F-FPIA uptake in human gliomas.

Secondary endpoint:

- Correlation of ^{18}F-FPIA uptake with tumour type and histological grade including O^6-methylguanine-DNA methyltransferase (MGMT) and isocitrate dehydrogenase (IDH) gene expression.

Tertiary endpoint:

- Correlation of ^{18}F-FPIA uptake with expression of genes responsible for metabolism on tissue obtained after surgery.
- Correlation of ^{18}F-FPIA uptake with tissue metabolites obtained after surgery.
- Correlation of ^{18}F-FPIA uptake with tissue proteins and phosphoproteins obtained after surgery.
- Correlation of ^{18}F-FPIA flux constants with histological grade.
- Correlation of ^{18}F-FPIA variables with structural and functional MRI variables including perfusion.

Selection of Subjects:

Inclusion Criteria:

Patients with radiological evidence of suspected cerebral glioma due for surgery or biopsy and with the following characteristics will be recruited:

1. Age ≥18
2. Tumour size at least 2 cm.
3. WHO performance status 0 – 2.
4. If female, the subject is either post-menopausal (at least 1 year), or surgically sterilized (has had a documented bilateral oophorectomy and/or documented hysterectomy for at least 2 years,), or if of childbearing potential, must have a negative urine beta human chorionic gonadotropin (β-HCG) pregnancy test done at initial screening and on the day of tracer administration. The result of the pregnancy test must be known before administration of ^{18}F-FPIA injection.
5. The subject is able and willing to comply with study procedures, and signed and dated informed consent is obtained.
6. The subject has a satisfactory medical history as judged by the investigator with no significant co-morbidities, physical examination, and vital signs findings during the screening period (from 21 days before administration).
7. The subject's clinical and laboratory tests are within normal limits and/or considered clinically insignificant.

Exclusion Criteria:

8. The subject has received any chemotherapy, immunotherapy, biologic therapy or investigational therapy within 14 days or five half-lives of a drug (whichever is longer) prior to the first dose of ^{18}F-FPIA injection. The subject is pregnant or lactating.
9. The subject is diabetic or has uncontrolled blood glucose or blood lipid levels (clinical decision by investigator), any other chronic illness that will preclude brief discontinuation of medication, or musculoskeletal condition that would not allow comfortable performance of a 66-minute study.
10. The subject has received another investigational radioactive tracer within 1 month before administration of ^{18}F-FPIA injection.
11. Anticoagulation therapy, prolonged prothrombin time, abnormal Allen's test.
12. Unsatisfactory renal function (eGFR<60)

Number of Patients Planned: 10

- In the event of unevaluable data, additional patients will be recruited to reach a total number of 10 complete patients.

Treatment of Subjects: Experimental imaging biomarker tracer:

- Subjects will receive a single I.V. bolus injection of ^{18}F-FPIA over a period of about 30 seconds. The tracer injection will be followed by a saline flush. Patients will receive a maximum ^{18}F activity of 370 MBq.

Statistical Methods and Planned Analysis:

- The study is an Observational Non-randomised Phase 2 study to determine the magnitude of the early steps of fatty acid oxidation in glioma using ^{18}F-FPIA PET/MRI. This is the first time we are using FPIA in patients (with glioma), thus we do not know *a priori* the magnitude of uptake. Statistical tests will use a 0.05 significance level and will be 2-sided unless otherwise noted. 10 evaluable patients will be recruited for the study with the Upper Confidence Interval of sensitivity to detect fatty acid metabolism set to $\geq$ 90%. All patients will undergo surgical resection or biopsy for histological confirmation of tumour grade.

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2. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
ARSAC	Administration of Radioactive Substances Advisory Committee
CT	Computed Tomography
ED	Effective Dose (i.e., the sum of risk weighted organ absorbed radiation dose used as a measure of stochastic radiation risk)
EC	Ethics committee
FDG	^{18}F – fluorodeoxyglucose
^{18}F	Fluorine with an Atomic Mass of 18
^{18}F-FPIA	^{18}F-fluoropivalate (^{18}F-fluoro-2,2-dimethylpropionic acid)
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
i.v	Intravenous
MBq	Megabecquerel
PET	Positron emission tomography
SAE	Serious adverse event
SUV	Standardized uptake value
MRI	Magnetic resonance imaging
NMR	Nuclear magnetic resonance
MS	Mass spectroscopy
TR	Time to repeat
TE	Time to echo

IDH	Isocitrate dehydrogenase
MGMT	O6-methly-guanly-methyl-transferase
LCMS	Liquid chromatography mass spectroscopy
eGFR	Estimated glomerular filtration rate

3. BACKGROUND

Glioma is the most common primary malignant brain tumour in adults and has an extremely poor prognosis. The two-year survival rate is a mere 26.5% for Glioblastoma (GBM), its most aggressive form despite treatment with radiotherapy and temozolomide. [1].

Gliomas like other tumours were originally thought to primarily metabolise glucose for energy production, however the reliance on glycolysis has recently been called into question. Lin et al. demonstrated that cells derived from human glioma, when cultured under optimal conditions expressed fatty acid oxidation enzymes and were dependent upon fatty acid oxidation for aerobic respiration and proliferation [2]. These findings were consistent with preclinical studies in which treatment of mice with etomoxir, an inhibitor of fatty acid oxidation demonstrated significantly reduced tumour growth and increased survival time [2]. Very recent work indicates a striking common metabolic phenotype of GBM and brain metastases to simultaneously oxidise acetate and glucose [3]. This adaptation may be important for meeting the high biosynthetic and bioenergetic demands of malignant growth. Furthermore, it has been shown that the key gene responsible for this phenotype, *ACSS2*, permits cells to exist under bioenergetic stress, which is a potential adaptation to therapy resistance [4]. Tumour cells, including brain tumour cells efficiently oxidize fatty acids synthesized from acetate to generate a substantial part of the cell's ATP requirement, as well as NADPH to buffer reactive oxygen species.

There is currently no method for quantifying tissue fatty acid oxidation in humans. Our group has characterized ^{18}F-fluoropivalate (^{18}F-FPIA; 3-^{18}F-fluoro-2,2-dimethylpropionic acid) as a tracer to report the early steps of fatty acid oxidation in non-clinical studies using PET imaging [4] [5]. ^{18}F-FPIA shows high contrast for imaging brain tumour xenografts implanted subcutaneously in mice [5], and orthotopically in the brain (unpublished) with the magnitude of tumour uptake equivalent to that of conventional tracers such as FDG but with significantly lower normal brain uptake giving a high signal to noise ratio enabling its use as suitable surrogate biomarker for fatty acid oxidation. Our current objective is to quantify the magnitude of early step fatty acid oxidation in human gliomas thought to possess enhanced fatty acid oxidation [3].

Combined hybrid PET/MRI technology permits simultaneous acquisition of PET imaging with conventional MRI sequences including T1, T2/FLAIR (with and without contrast) and perfusion (DSC-MRI). Patients with Glioma will all undergo routine biopsy or surgery following imaging and so we will have the opportunity to correlate the degree of fatty acid oxidation with tumour grade and genes involved in metabolism. Further tissue analysis will involve liquid chromatography mass spectroscopy-metabolomics, protein, and phospho-protein analysis. Mathematical modelling of tissue and blood data will be conducted to permit derivation of flux constants describing delivery and retention of the radiotracer.

4. RATIONALE FOR CURRENT STUDY

4.1 Aim:

The aim of this study is to quantify the degree of early step fatty acid oxidation in gliomas as imaged by ^{18}F-FPIA PET/MRI.

4.2 Hypothesis:

We hypothesise that FPIA uptake will be higher in high-grade gliomas compared to lower grade gliomas, in keeping with a higher propensity of high grade tumours to generate ATP and NADPH via fatty acid oxidation under bioenergetic stress.

4.3 Statistical Design:

The study is an Observational Non-randomised Phase 2 study to determine the magnitude of the early steps of fatty acid oxidation in glioma using ^{18}F-FPIA PET/MRI. This is the first time we are using FPIA in patients (with glioma), thus we do not know *a priori* the magnitude of uptake. Statistical tests will use a 0.05 significance level and will be 2-sided unless otherwise noted. 10 evaluable patients will be recruited for the study with the Upper Confidence Interval of sensitivity to detect fatty acid metabolism set to $\geq$ 90%. All patients will undergo surgical resection or biopsy for histological confirmation of tumour grade.

5. STUDY OBJECTIVES AND ENDPOINTS

5.1 Primary Objective:

- Determine the magnitude of the early steps of fatty acid oxidation in glioma using ^{18}F-FPIA PET/MRI.

5.2 Secondary objective:

- Compare magnitude of tracer-derived biochemical uptake measure with tumour grade.

5.3 Tertiary objective:

- Compare magnitude of tracer-derived biochemical uptake measure with ex vivo gene expression specifically for metabolism.
- Compare magnitude of tracer-derived biochemical uptake measure with ex vivo tissue metabolite analysis using Mass Spectrometry (MS).
- Compare the magnitude of tracer-derived biochemical uptake measure with ex vivo protein and phosphoprotein analysis from the major cell proliferation and survival pathways including phospho ERK 1/2 and pSer641.
- Comparison of flux constants derived from mathematical modelling of ^{18}F-FPIA PET/MRI data with tumour grade, contrast enhancement and perfusion.

6. STUDY ENDPOINTS

6.1 Primary Endpoint:

- Quantitative measurement of ^{18}F-FPIA uptake in human gliomas.

6.2 Secondary endpoint:

- Correlation of ^{18}F-FPIA uptake with tumour type and histological grade including O^6-methylguanine-DNA methyltransferase (MGMT) and isocitrate dehydrogenase (IDH) gene expression.

6.3 Tertiary endpoint:

- Correlation of ^{18}F-FPIA uptake with expression of genes responsible for metabolism on tissue obtained after surgery.
- Correlation of ^{18}F-FPIA uptake with tissue metabolites obtained after surgery.
- Correlation of ^{18}F-FPIA uptake with tissue proteins and phosphoproteins obtained after surgery.
- Correlation of ^{18}F-FPIA flux constants with histological grade.
- Correlation of ^{18}F-FPIA variables with structural and functional MRI variables including perfusion.

7. STUDY OUTLINE

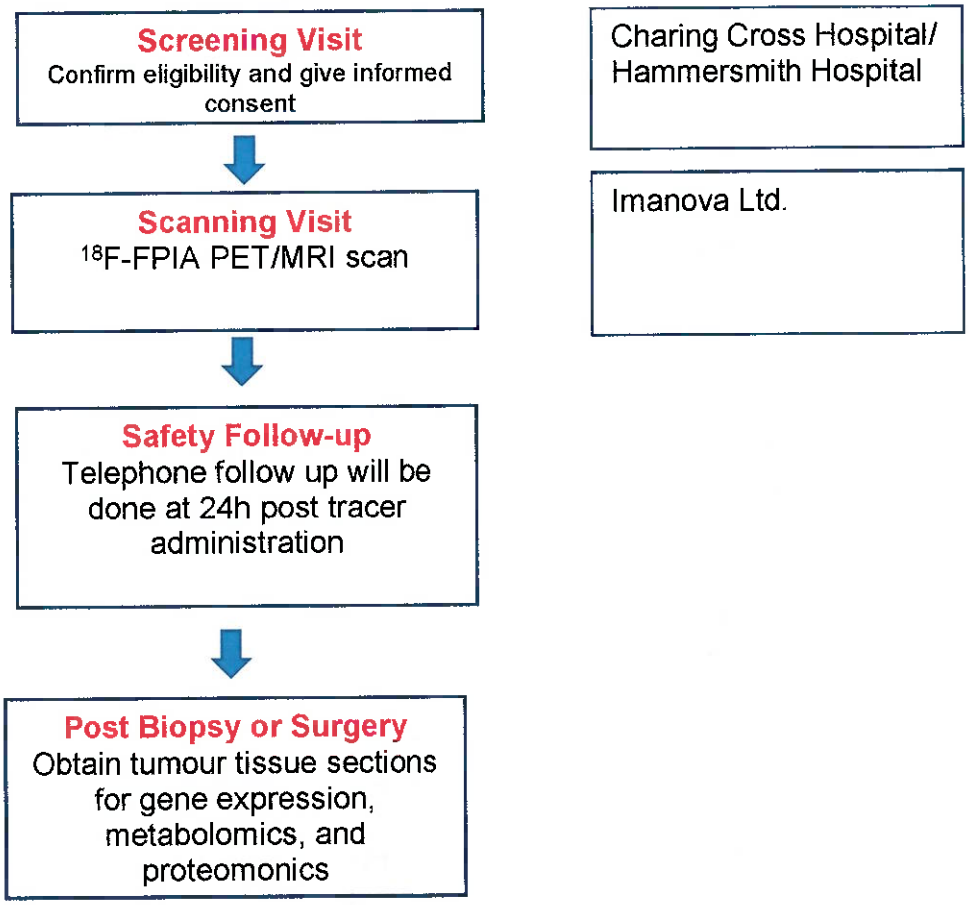
7.1 Study Design

10 evaluable patients with suspected cerebral glioma on previous MRI who are due to undergo surgical resection or biopsy will be enrolled into the study. The patients invited to participate in the study will provide full consent, but will only undergo 18F-FPIA PET/MRI imaging once they have satisfied the inclusion and exclusion criteria in section 8. Once these have been satisfied, eligible patients will proceed to 18F-FPIA PET/MRI.

On the day of imaging the patients will undergo a blood test to measure plasma concentrations of carnitine. During the scan, a single dose of ^{18}F-FPIA (maximum, 370 MBq) IV will be administered to the participant followed by a whole brain dynamic PET/MRI scan over 66 minutes. During the MRI sequences, the patient will receive an additional IV bolus of Gadolinium contrast medium administered through a peripheral venous cannula. Arterial blood sampling through a peripheral arterial line will be performed to determine the concentration of radiotracer within arterial plasma. All the patients that are enrolled into the study will undergo biopsy or surgical resection as part of their routine clinical care, from which their tumour grade will be confirmed; we will obtain tissue from these procedures to perform metabolomics, genomics and proteomics. Surgery or biopsy will be performed typically within 2 weeks but no later than 3 months'. In anticipation of any transformation whilst patients are awaiting surgery, we will record the exact time between imaging and surgery for each patient.

7.2 STUDY SCHEDULE

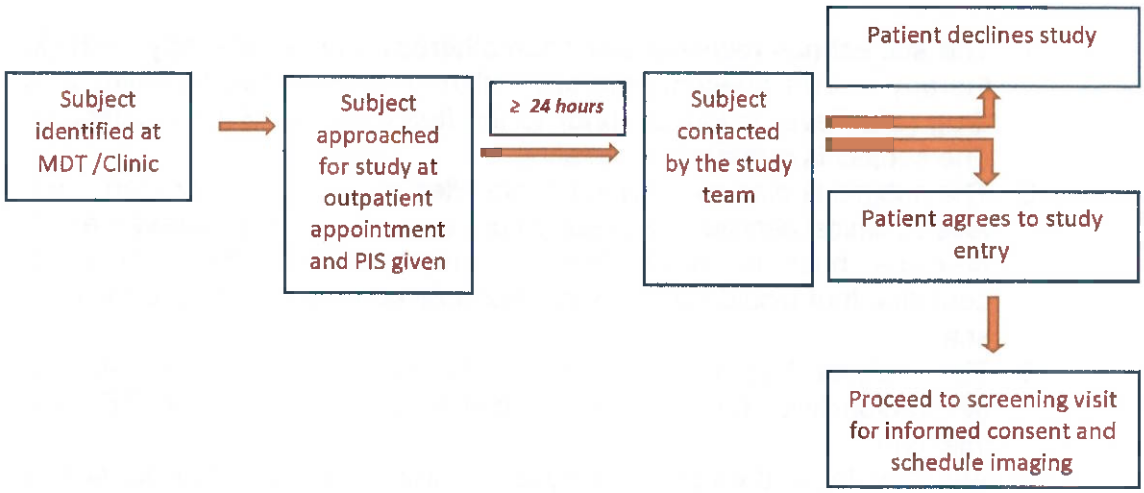
Figure 1.



7.3 Patient Identification and recruitment

Potentially eligible patients will be identified by their clinician at Charing Cross Hospital. An explanation of the study will be given by the treating clinician and they will be provided with the Patient Information Sheet (PIS) to take away. Patients will have a minimum of 24 hours to consider their participation in the trial before any trial related activities take place. After 24 hours and following verbal consent from the patient, the potential participant will be contacted by a member of the study team to answer any questions they may have regarding the study. Should they wish to participate, they will then be invited to attend a screening visit up to 21 days before tracer administration and imaging.

Figure 2. Recruitment pathway



8. ELIGIBILITY

8.1 Inclusion Criteria:

Patients with radiological evidence of suspected cerebral glioma due for surgery or biopsy and with the following characteristics will be recruited:

1. Age ≥18
2. Tumour size at least 2 cm.
3. WHO performance status 0 – 2.
4. If female, the subject is either post-menopausal (at least 1 year), or surgically sterilized (has had a documented bilateral oophorectomy and/or documented hysterectomy for at least 2 years), or if of childbearing potential, must have a negative urine beta human chorionic

gonadotropin (β-HCG) pregnancy test done at initial screening and on the day of tracer administration. The result of the pregnancy test must be known before administration of ^{18}F-FPIA injection.

5. The subject is able and willing to comply with study procedures, and signed and dated informed consent is obtained.
6. The subject has a satisfactory medical history as judged by the investigator with no significant co-morbidities, physical examination, and vital signs findings during the screening period (from 21 days before administration).
7. The subject's clinical and laboratory tests are within normal limits and/or considered clinically insignificant.

8.2 Exclusion Criteria:

1. The subject has received any chemotherapy, immunotherapy, biologic therapy or investigational therapy within 14 days or five half-lives of a drug (whichever is longer) prior to the first dose of ^{18}F-FPIA injection. The subject is pregnant or lactating.
2. The subject is diabetic or has uncontrolled blood glucose or blood lipid levels (clinical decision by investigator), any other chronic illness that will preclude brief discontinuation of medication, or musculoskeletal condition that would not allow comfortable performance of a 66-minute scan.
3. The subject has received, or is scheduled to receive, another IMP/radioactive tracer 1 month before administration of ^{18}F-FPIA injection.
4. Anticoagulation therapy, prolonged prothrombin time, abnormal Allen's test.
5. Unsatisfactory renal function (eGFR<60)
6. The subject has non-MRI compatible devices (a pacemaker, an implantable cardioverter-defibrillator (ICD), a nerve stimulator, a cochlea implant and a drug pump) or implanted material (e.g. non-MRI compatible sternal wires, biostimulators, metals or alloys).

9. COMPLETION, WITHDRAWAL AND TERMINATION CRITERIA

9.1 Subject completion

A completed subject is one who has completed all imaging sequences of the combined ^{18}F-FPIA PET/MRI protocol.

9.2 Rules for subject withdrawal

There are no formal withdrawal criteria for this study. During the conduct of the study, the study team will review the safety data for trends and signals that would indicate the need for withdrawal of a subject.

In accordance with the Declaration of Helsinki, each subject is free to withdraw from the study at any time. Investigator(s) also have the right to withdraw subjects from the study in the event of illness, AEs, or other reasons concerning the health or well-being of the subject, or in the case of lack of co-operation. Should a subject decide to withdraw after administration of the tracer, or should the investigator decide to withdraw the subject, all efforts will be made to complete and report the observations up to the time of withdrawal as thoroughly as possible. A complete final evaluation at the time of the subject's withdrawal will be made and an explanation given of why the subject is withdrawing or being withdrawn from the study.

The reason for non-completion and the date and time of the last contact with the subject must be noted in the research documentation. If the reason for withdrawal is a clinical AE or an abnormal laboratory result, monitoring will continue until the outcome is evident. The specific event or test result(s) must be recorded in the research documentation. In the case of subject withdrawal before or after dosing (i.e, non-evaluable subjects) additional subjects may be enrolled to reach a total of 10 evaluable subjects.

9.3 Rules for terminating study

The sponsor reserves the right to terminate the study at any time. Prior to dosing of the first subject, the study may be terminated by the sponsor and the investigator without consultation with the Administration of Radioactive Substances Advisory Committee (ARSAC) holder and Ethics Committee. The ARSAC holder, Ethics Committee and Health Research Authority (HRA), must be promptly notified that the study will no longer be taking place and provided with a detailed written explanation. Once dosing with radiopharmaceutical has begun, the study may only be terminated if a careful review of the overall risk benefit analysis demonstrates that the assumptions have changed and that the overall balance is no longer acceptable. The investigators will temporarily halt the study if there has been a SAE which has been judged to be related to the study dosing. In these circumstances termination can only take place with the agreement of the Sponsor, ARSAC holder and the Ethics Committee.

If it becomes necessary to consider termination of the study after dosing has begun, dosing may be suspended pending discussion between the sponsor, the investigator, the ARSAC holder and the Ethics Committee. Dosing may always be immediately suspended for safety reasons.

10. STUDY PROCEDURES

The patient procedures are summarized in Table 1 below.

Table 1. Study Schedule of Events:

	Screening	Pre-PET/MRI dose(same day)	Dose	+ x min	+ x min	+ x min	+ x min	+ x min	Telephone follow up 24 hours post tracer injection	Post biopsy or surgery, obtain tumour tissue sections for grading, gene expression, metabolomics, and proteomics
Informed Consent	•									
Entry Criteria	•									
Demographic Information	•									
Medical History	•									
Prior/Concomitant Medication [1]	•	•								
Physical Exam including Allen's Test [1]	•	•								
Blood test for haemaglobin, clotting profile and renal function[2]	•									
Carnitine blood test		•								
Pregnancy Test [1]	•	•								
Vital Signs [1]	•	•								
^{18}F-FPIA injection			•							
Gadolinium contrast injection 0.2ml/kg				•	•					
Simultaneous PET/MRI Imaging*			continuous							
Arterial Blood Activity Counting			•	•	•	•	•	•		
Adverse events			•	•	•	•	•	•		

- [1]If screening and PET/MRI are on same day – procedures do not have to be repeated.
- [2]If result available from routine test within 30 days of ^{18}F-FPIA injection, this does not need to be repeated (determined by investigator)
- Note: sampling times may be changed if necessary for scientific or logistical reasons but will not exceed the volume stated.
- *For further details regarding the MRI protocol see Section 11.4

11. IMAGING PROTOCOL

11.1 Radiopharmaceutical

The ^{18}F-FPIA radiotracer will be made to GMP standards at Imanova Limited, which is GMP certified.

11.2 Imaging protocol

- ^{18}F-FPIA PET/MRI imaging will be carried out at the Hammersmith Hospital site (within Imanova Limited, Du Cane Road, London
- A peripheral venous cannula will be inserted within the arm for radiotracer and MRI contrast injections.
- An arterial cannula will be inserted in the radial artery prior to the start of the scan (after an Allens' test to ensure satisfactory ulnar circulation) under local anaesthetic to allow arterial blood sampling during the scan. Continuous arterial blood sampling at 5mL/min will be performed for the first 10 minutes. Discrete blood samples from baseline to 60 minutes post administration will also be taken for analysis of 18F metabolites. The total blood volume required for all analyses in the study will not exceed 200ml. ^{18}F activity will be measured using a gamma counter according to the working instructions at the site. Volumes will be adjusted to avoid counter saturation.
- Simultaneous ^{18}F-FPIA PET and MRI whole brain imaging (vertex to C1 vertebral body) will be performed,
- Following completion of imaging venous and Arterial cannulae will be removed at the end of the scan and patient will be discharged.
- The patient will be contacted via telephone 24 hours following radiotracer injection, to enquire about any adverse health problems following the scan.

11.3 PET Protocol

- For the PET scan, a maximum of 370 MBq ^{18}F-FPIA will be injected followed by a saline flush.
- PET data will be acquired as a single-bed position dynamic imaging scan of approximately 66 minutes
- Discrete arterial samples totalling no more than 200 ml for metabolite analysis and activity counting will be taken during the scan.

11.4 MRI Protocol

The MRI protocol will include the following sequences:

- Sequences for attenuation correction of PET data (DIXON, zero-TE)
- Volumetric T1-weighted images, pre- and post-contrast administration
- Volumetric T2
- Dynamic susceptibility contrast perfusion (DSC)
- Dynamic contrast enhanced perfusion (DCE)

The exact image acquisition parameters for sequences (e.g. TE, TR, flip angle) are hardware dependent and vary between scanner manufacturers and models, and are therefore not specified here. DWI sequences will be acquired prior to contrast agent administration.

11.5 Radiation Dosimetry

Table 2. Radiation dose estimation:

Tracer	^{18}F-FPIA PET
Maximum ^{18}F-FPIA injected activity *per visit*	370 MBq
Conversion factor (mSv/MBq)	0.0187
Total effective dose (mSv)/ patient	6.9 mSv

In aggregate, the total effective dose for each patient in this study is 6.9 mSv. There is no ionizing radiation from the MR component of the study.

11.6 Data Analysis

Raw PET dynamic attenuation corrected images will be reconstructed. Decay corrected images will then be viewed using Analyze®, Hermes or other appropriate software. Next, the summed images from 15 min to 65 min will be obtained and regions of interest (ROI) will be drawn around the tumour and normal tissues to derive semi-quantitative uptake parameters: SUV (standardized uptake value) normalised to body weight (bw). The average and maximum SUV at 60 min ($SUV_{60,av}$, and $SUV_{60,max}$) will be calculated. Arterial blood samples counted for tracer activity and metabolites of ^{18}F-FPIA will be used to generate the blood input function used to derive the uptake tracer parameters (image derived input function

will also be used).

11.7 Metabolite analysis

The discrete arterial samples taken during the scan will be analysed. Total blood radioactivity will be measured by gamma counting and plasma ^{18}F-FPIA parent fraction will be determined by reverse-phase high-performance liquid chromatography with radiochemical detection.

11.8 Kinetic analysis

The kinetics of ^{18}F-FPIA will be estimated from spectral analysis-derived unit impulse response function (IRF) and Patlak analysis-derived net irreversible plasma to tumour transfer constant (Ki, ml of plasma·s−1·ml tissue−1), as well as compartmental analysis-derived ^{18}F-FPIA delivery (K1, ml of plasma·s−1·ml tissue−1) and retention (k3, ml of plasma·s−1). For comparison, tracer retention will also be estimated from SUV analysis (ratio of SUV at 60 min relative to 5 min).

11.9 Tissue Analysis

All patients enrolled into the study, will have their routine biopsy or surgery tissue made available for analysis. We will seek data from routine clinical tissue assays including MGMT, IDH-1 status used for confirming tumour grade.

Following this we will perform our own analysis on tissue including gene, protein, and phospho-protein analysis. Illumina RNA Seq method or similar will be used for gene expression analysis. An automated reverse-phase protein array (RPPA) platform offering detection of approximately 150-170 protein and phospho-proteins from the major cell proliferation and survival pathways (including phospho-ERK 1/2, GYS1-pSer641) will be employed for protein/phospho-protein analysis. Tumour tissue will be obtained for NMR and Mass Spectrometry metabolomics at the Imperial College Biomedical Research Centre (BRC) Clinical Phenome Centre.

11.10 MRI analysis

MRI structural imaging sequences will be used for motion correction of PET data and to define tumour volumes using semi-automated intensity based segmentation algorithms such as those available in the software 'Jim'. Perfusion data will be analysed using custom in-house image analysis tools and other software tools such as MATLAB, FSL, Nordic Neurolab and Tarquin. MRI and PET imaging

parameters will be combined to develop novel biomarkers which can be used for tumour characterisation and assessment of treatment response.

12. COMPLICATIONS AND RADIATION DOSE

12.1 Complications

There are no immediate complications anticipated of the PET/MRI scan except for a potential mild bruising at the site of insertion of the peripheral cannulas which should resolve in 1-2 weeks. Scans will be stopped at any time during the procedure if the subject is unable to tolerate it.

12.2 Radiation dose

The total effective dose (ED) received from the PET/MRI scan is estimated to be 6.9 mSv. This is similar to the exposure to naturally occurring background radiation for the general population of the UK over a period of 3 years. This exposure should be seen in context of the patients in this study being repeatedly exposed to radiation from X-rays, CT scans and radiotherapy as part of routine care.

12.3 Treatment of subjects

The investigators are responsible for ensuring that deliveries of the tracer and study material are correctly received, recorded, handled and stored safely and properly, in accordance with regulatory guidelines (ICH-GCP and Good Manufacturing Practice (GMP) and used in accordance with this protocol.

13. ADVERSE EVENTS

13.1 Definitions

Adverse Event (AE Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any serious adverse event that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- **Requires hospitalization or prolongation of existing inpatients' hospitalization.**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

13.2 Reporting Procedures

All adverse events up to 24 hours post study tracer injection should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

13.3 Non serious AEs

All such events, whether expected or not, should be recorded.

13.4 Serious AEs

An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to a pre-existing condition, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the Research Ethics Committee where in the opinion of the Chief Investigator, the event was:

- 'related', i.e. resulted from the administration of any of the research procedures; and
- 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non- IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs. Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

14. REGULATORY ISSUES

14.1 Pre-Trial Requirements

Treatment within this study will only start if the following pre-requisites have been fulfilled and documentation is at hand:

- Signed copy (original) of the approved protocol
- Ethics Committee (EC) approval of the protocol, consent form and information sheet
- Approval / notification of local authorities

The Investigator will hold an Investigator's File containing the relevant study documents.

14.2 General Legal Requirements

The study will be conducted in agreement with the following directives and guidelines:

- The Declaration of Helsinki (version of Edinburgh, Scotland, October 2000)
- The respective Guidelines of the European Community: Guideline for Good Clinical Practice (Note for Guidance on Good Clinical Practice / ICH E6) and Statutory Instrument 2004 No. 1031 - The Medicines for Human Use (Clinical Trials) Regulations 2004 as amended.

All clinical work conducted under this protocol will be subject to Good Clinical Practice rules. This includes an inspection by health authority representatives at any time. The Investigator agrees to the inspection of study-related records by health authority representatives and/or the sponsor.

14.3 Handling and Quality Assurance

The Chief Investigator will be responsible for the processing and quality control of the data. The handling of data, including data quality control, will comply with all applicable regulatory guidelines. All study documentation at the investigator site and sponsor site will be archived in accordance with the sponsor's quality standards.

14.4 Protection of Subjects

14.4.1 Ethics Committee

All patients will be recruited strictly according to protocol following the principles of the Declaration of Helsinki. All inclusion criteria would be satisfied before enrolling patients. The protocol and a copy of the subject information and informed consent form will be submitted to the competent EC, Health Research Authority (HRA), Sponsor and Local Research & Development departments. Written approval of the protocol and the proposed information and consent form will be obtained prior to the start of the trial.

14.4.2 Subject Informed Consent

It is the Investigator's responsibility to explain to each subject the study procedure, potential benefits and hazards of trial participation, the right to withdraw from the study at any time, and to obtain written informed consent prior to any study-specific procedures. The subject, the subject's legally authorized representative, or both will be given a copy of the signed and dated Informed Consent Form.

14.4.3 Direct access to source data/documents

The monitor(s), auditor(s), authorised personnel of the sponsor, and health authority inspector(s) or their agents will be given direct access to source data and documentation (e.g., medical charts/records, laboratory results, printouts, PET/MRI scans etc.) for source data verification, provided that subject confidentiality is maintained in accordance with local requirements.

14.5 Confidentiality

14.5.1 Data

Confidentiality of subjects including personal information and medical records will be maintained to the extent permitted by law under the Data Protection Act. Personal medical information may be scrutinised for the purpose of verifying patient data. Representatives of Imanova Ltd and authorised persons on behalf of the NHS sponsor and/or regulatory bodies will be able to have direct access to original relevant medical records for the verification of clinical study procedures and quality assurance purposes. No information will be disclosed to anyone, other than these bodies, other than that required for the purposes of product registration, medical research, or as otherwise required by law.

Subjects will be given a unique identity code for the purpose of the study. Data and

images obtained from scans may be used in an anonymous form for future research including that carried out by commercial healthcare companies. Subject identity will remain confidential in any subsequent publications.

The subject's GP will be informed of their participation in the study. Scan results will be communicated to the Doctor responsible for the subject's brain tumour treatment. Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

14.6 Samples

Blood samples taken during the study will be analysed at Imperial College London, Imperial College NHS Trust Hospital, and Imanova Ltd depending on the specific test, with exception to the carnitine assay, which will be analysed at an external third party NHS laboratory. They will be destroyed once the tests are done and will not be stored for future use.

The brain tissue sample will be collected through the tissue bank neuropathology subcollection service. Samples will be analysed within laboratories at Imperial College London and MD Anderson (Texas, USA). Following completion of analysis all samples will be returned back to the tissue bank at Imperial College NHS Trust Hospital.

If the subject withdraws from the study, samples already taken as part of the research will be destroyed if the subject wishes, but data collected up to the withdrawal will be used.

Patients' identification data will be required for the registration process. The Study Coordination Centre will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act. Image datasets will be allocated a unique identification code.

14.7 Protocol Amendments

The protocol must be read thoroughly and the instructions must be followed exactly. Amendments to the protocol must be reviewed by the sponsor and before submission to the relevant authorities. Necessary approvals must be in place before it can be implemented. Urgent safety measures may be implemented immediately, but sponsor, ethics and HRA approval should be sought as soon as possible after the event.

14.8 Premature Termination of the Trial

The Investigator reserves the right to terminate the trial for well-documented reasons. Instructions will be provided in a separate document should it be determined that assessments beyond those defined by the protocol are required.

Further recruitment of subjects will not take place under the following conditions:

- Premature termination of the trial.
- Procedure-related events, i.e., the recruitment rate is too low or the number of dropouts for administrative reasons is too high.

15. STUDY MONITORING

15.1 Indemnity

Imperial College London holds Public Liability ("negligent harm") and Clinical Trial ("non- negligent harm") insurance policies, which apply to this trial.

15.2 Sponsor

Imperial College London will act as the main sponsor for this study. Delegated responsibilities will be assigned to the NHS trust taking part in this study.

15.3 Funding

This study is funded by a Medical Research Council (MRC) programme grant awarded to Imperial College London.

15.4 Audits and inspections

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

15.6 Disclosures

There are no personal or departmental financial interests or other similar benefits relating to this project.

15.7 Publications

Publication of the results of the study, whether in whole or in part, shall be within the sole and absolute discretion of the Investigator.

16. References

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